SUBJECT: Abbreviated Ecological Hazard Assessment for J16-3

FROM: Alie Muneer
Physical Scientist (Environmental)
Assessment Branch 2
Risk Assessment Division (7403M)

THRU: Iris Camacho
Acting Branch Chief, AB2

TO: Gwendolyn McClung Ph.D., Technical Integrator
RAD/AB3 (7403M)

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********** [redacted] **********

I. INTRODUCTION

The Agency has received a Microbial Commercial Activity Notice (MCAN) from DSM Bio-based Products and Services (DSM Nutritional Products) for the submission [redacted].

Although *S. cerevisiae* is one of the ten microorganisms eligible for the 5(h)4 Tiered Exemptions from MCAN reporting and the submission claims that their strain meets all the criteria for the introduced genetic material for the Tier I Exemption, the company has chosen to submit this strain for an MCAN review because it is intended for use in, and thus transport to, multiple ethanol production facilities in the U.S. The submitter wishes to ease the administrative burden of the Tier 1 application process that would be required from all future [redacted]. In addition, the transport of the microorganism to various facilities is outside the realm of the Tier I Exemption. Thus, the company is submitting this single MCAN for a thorough review of the intergeneric microorganism given its use at a number of different production facilities.

II. TAXONOMY AND CHARACTERIZATION

A. Recipient Microorganism

Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as baker's yeast or brewer's yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages. The risk assessment of *S. cerevisiae* for the 5(h)(4) Tiered Exemptions Final Risk Assessment for *S. cerevisiae:* (

Although it is associated with human activity from bread baking and fermentation of alcoholic beverages, *S. cerevisiae* is widespread in nature. It has been recovered from a variety of sites such as soils, sediments, and plant material under different ecological conditions. *S. cerevisiae* is frequently recovered from fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. In the environment, yeasts can be dispersed by insects, particularly fruit flies (Gilbert, 1980).

B. Donor Microorganism

The donor microorganisms are

The submission microorganisms contain the xylose isomerase gene (*xylA*) from *Piromyces* sp. E2 which enable utilization of the 5-C sugar xylose found in hemicellulose. This microorganism is an anaerobic chytridiomycete fungus that produces ethanol via pyruvate using a pyruvate:formate lyase and an alcohol dehydrogenase (Boxma et al., 2004). *Piromyces* sp. E2 comes from feces of an Indian elephant and xylose isomerase is used by industry to produce high fructose corn syrup.

Also introduced for utilization of the 5-C sugar arabinose were the *araA* (arabinose isomerase), *araB* (ribulose kinase), *araD (*ribulose-5-phosphate 4-epimerase) and *acdH* (acetylating acetaldehyde dehydrogenase) genes from the bacterium *Lactobacillus plantarum.* This bacterium naturally occurs in the human gastro-intestinal tract and in a range of fermented food products, such as sauerkraut, olives, wine, dry sausage and probiotics and is traditionally used in sourdough fermentations (La Marta, 2016).

The submitters introduced a putative arabinose transporter (Ara*T*), Pc20g01790 from *Penicillium chrysogenum*, sequence optimized for expression in *S. cerevisiae*. *P. chrysogenum* has a long-standing tradition as industrial microbe and is used to produce penicillin and other antibiotics since its discovery by Alexander Fleming in 1928 (La

Marta, 2016). *P*. *chrysogenum* is found in salted foods and is common in temperate and subtropical regions.

The eutE-gene from *Escherichia coli* was introduced in *S. cerevisiae* recipient strain. *E. coli* is found in the lower intestine of warm-blooded organisms and most strains are harmless. The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K_2 and preventing colonization of intestine with pathogenic bacteria. *E. coli* is released to the environment through deposition of fecal material.

III. ECOLOGICAL HAZARDS

A. Recipient Microorganism

The recipient microorganism does not pose any pathogenicity/toxicity concerns to plants or animals. It is a benign yeast with a long history of safe use that is ubiquitous in the environment. The risk assessment of *S. cerevisiae* for the 5(h)4 Tiered Exemption stated that there are low ecological hazards associated with this microorganism.

B. Submission Microorganism

The introduced intergeneric genes for xylose (*xylA*) utilization, arabinose utilization (*araA, araB,* and *araD*), high affinity arabinose transporter (*Pc20g01790*) and acetylating acetaldehyde dehydrogenase (*acdH*) do not pose any concerns for pathogenicity/toxicity of the submission microorganisms. They merely enable the yeast to use the 5-C sugars that are found in hemicellulose. These genes were introduced to [redacted] from plant material containing both cellulose and hemicellulose. Ethanol production is an existing trait in the *S. cerevisiae.*

The potential for horizontal gene transfer of xylose isomerase (*xylA)*, arabinose isomerase (*araA*), ribulose kinase (*araB*) and ribulose-5-phosphate epimerase (*araD*) to occur between eukaryotic cells to prokaryotic cells is low due to the rapid degradation of DNA in the environment, low percentage of competent bacteria that will be naturally present in the environment and low transformation efficiency of competent bacteria (La Marta, 2016).

Although the strain may survive if inadvertently released into the environment, there would be no ecological concerns. *S. cerevisiae* is widespread in the environment in sugar-rich niches. The enhanced [redacted] capability by enabling utilization of xylose and arabinose does not pose ecological concerns. The submission microorganism does not contain any ARM (antibiotic resistance marker) genes. Antibiotic resistance markers were used in the development of parental strains but were later removed. The submission microorganism is naturally susceptible to antibiotics and to anti-fungals.

IV. CONCLUSIONS

There are low ecological hazards associated with the use of the production strain of *S. cerevisiae* for [redacted]. The recipient microorganism does not pose ecological concerns, nor does the introduced genetic material.

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